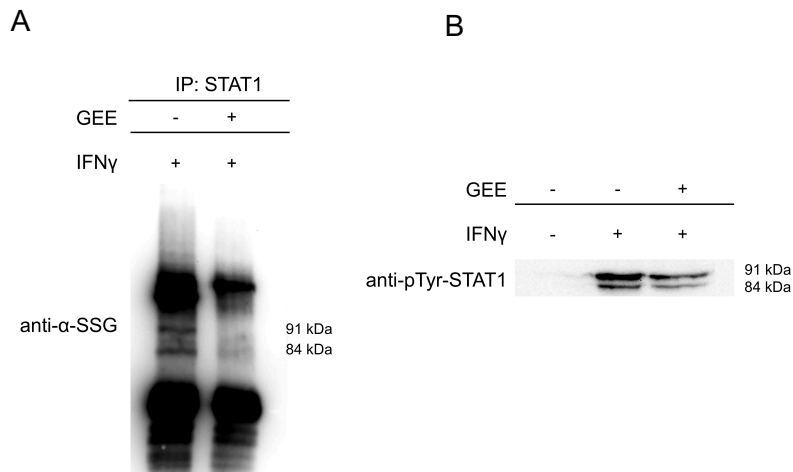


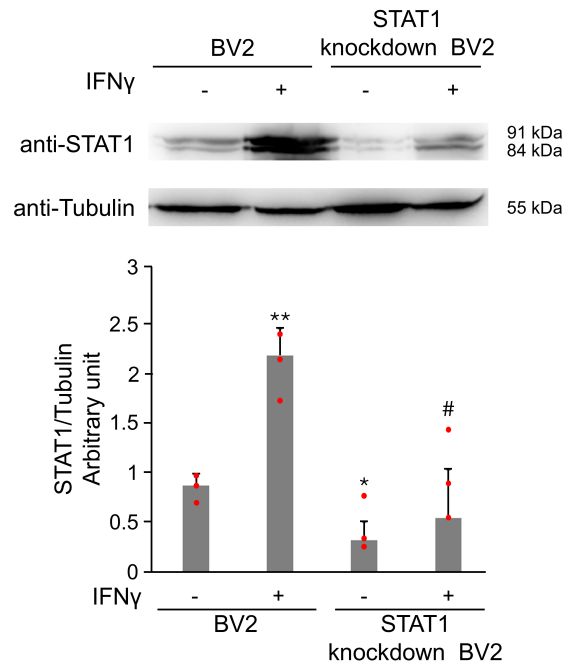
Supplementary material

ROS-driven STAT1 S-glutathionylation sustains IFN γ signaling and pro-inflammatory microglial polarization

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Supplementary 1. GEE pretreatment reduces IFN γ -induced STAT1 S-glutathionylation in BV2 cells. A) Total protein extracts from BV2 cells pretreated or not with 1 mM GEE (overnight) and then stimulated with 20 ng/mL IFN γ for 15 minutes were subjected to immunoprecipitation with anti-STAT1 antibody. Immunoprecipitated STAT1 (IP: STAT1) was analyzed by Western blot under non-reducing conditions using anti-SSG antibody. **B)** Total protein lysates from the same cells were reserved before pull-down (input) and analyzed by Western Blot with anti-phospho-Tyr701 STAT1 antibody. GEE pretreatment markedly reduced IFN γ -induced STAT1 S-glutathionylation and TYR phosphorylation.



Supplementary 2. Lentiviral transduction of BV2 cells allows STAT1 silencing. Parental and STAT1 knockdown in BV2 cells were exposed to 20 ng/mL of IFN γ for 18 hours, and total protein extracts were analysed by Western Blot with anti-STAT1 and with anti-actin antibodies. Densitometric analysis revealed decreasing levels of STAT1 in both untreated and IFN γ -treated STAT1 knockdown in BV2 cells with respect to parental cells.